Listing of Claims

1. (Original) A method for generating a vector for conditional knockout of a gene in a cell, comprising

using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome, wherein a vector comprises the bacterial artificial chromosome;

excising the nucleic acid encoding the selectable maker with a first recombinase specific for the first recombining sites, wherein a single first recombining site remains in the gene;

using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of second recombining sites and a first recombining site into a second site in the gene; and

excising the nucleic acid encoding the selectable marker with a second recombinase specific for the second recombining sites, wherein two first recombining sites remain in the gene following excision of the nucleic acid encoding the selectable marker, wherein recombination of the two first recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein,

thereby generating the vector for conditional knockout of the gene in the cell.

2. (Original) The method of claim 1, wherein the cell comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and wherein using homologous recombination comprises activating the de-repressible promoter, thereby inducing the expression of Beta and Exo.

3. (Original) The method of claim 2, wherein either the first recombining sites or the second recombining sites comprise a LoxP site.

4. (Original) The method of claim 2, wherein the first recombining sites comprise a LoxP site, and the second recombining sites comprise a frt site.

5. (Original) The method of claim 2, wherein the first recombining sites comprise a frt site, and the second recombining sites comprise a LoxP site.

6. (Original) The method of claim 2, wherein using homologous recombination to insert the nucleic acid encoding the selectable marker flanked by the pair of first recombining sites comprises

introducing a double-stranded vector comprising the nucleic acid encoding the selectable marker flanked by the pair of first recombining sites into a host cell comprising a nucleic acid sequence encoding Exo, Beta and Gam, operably linked to a de-repressible promoter, wherein the vector further comprises a sufficient number of nucleotides homologous to the bacterial artificial chromosome flanking each of the pair of first recombining sites to achieve homologous recombination;

selecting a host cell in which homologous recombination has occurred.

7. (Original) The method of claim 2, wherein the cell further comprises an inducible promoter operably linked to a nucleic acid encoding the first recombinase, and wherein excising the nucleic acid encoding the selectable maker comprises inducing the expression of the first recombinase.

8. (Original) The method of claim 7, wherein the first recombinase is Cre.

9. (Original) The method of claim 7, wherein the first recombinase is Flpe.

10. (Original) The method of claim 7, wherein the cell is a bacterial cell.

11. (Original) The method of claim 7, wherein the cell is a eukaryotic cell.

12. (Original) The method of claim 2, wherein the cell comprises an inducible promoter operably linked to a nucleic acid encoding the second recombinase, and wherein excising the nucleic acid encoding the selectable marker comprises inducing the expression of the second recombinase.

13. (Original) The method of claim 1, wherein the selectable marker confers resistance of the cell to an antibiotic.

14-21 (Canceled).

22. (New) The method of claim 2, wherein the de-repressible promoter is pL.

23. (New) The method of claim 6, wherein the de-repressible promoter is pL.